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EXAMINER	
STAPLES, MARK	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/508,799	JOHANSEN, JACK T	
	Examiner	Art Unit	
	MARK STAPLES	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 July 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-4 and 6-28* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4 and 6-28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 07/20/2009 has been entered.

2. Applicant's amendment of claims 1, 4, and 28 and cancellation of claim 5 in the paper filed on 07/20/2009 is acknowledged.

Claims 1-4 and 6-28 are pending and at issue.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Rejections that are Moot / Withdrawn

Canceled Claim Rejection Moot / Withdrawn

3. The rejection of canceled claim 5 is moot and therefore is withdrawn.

Claim Rejections Withdrawn - 35 USC § 103(a)

4. The rejection of claims 3-4 and 24 under 35 U.S.C. 103(a) as being unpatentable over Berglund et al. as applied to claim 1, and further in view of Bloch (United States Patent 5,856,192 issued January 5, 1999) is withdrawn. Applicant's arguments have been considered but are moot in view of the new ground(s) of rejection. The new rejection which appears below and addresses Applicant's concern that Examiner also presented teachings of Bloch which apply to claims 1, 2, and 28 which claims were already rejected as being anticipated by the teachings of Berglund et al.

5. The rejection of claim 9 under 35 U.S.C. 103(a) as being unpatentable over Berglund et al. as applied to claims, and further in view of Bloch (1999) and Crane et al. (1992, previously cited) is withdrawn. Applicant's arguments have been considered but are moot in view of the new ground(s) of rejection.

6. The rejection of claim 15 under 35 U.S.C. 103(a) as being unpatentable over Berglund et al. as applied to claims 1 and 7, and further in view of Asteriadis et al. (1976, previously cited) is withdrawn. Applicant's arguments have been considered but are moot in view of the new ground(s) of rejection.

7. The rejection of claims 22, 23, and 26 under 35 U.S.C. 103(a) as being unpatentable over Berglund et al. as applied to claim 1 and further in view of Fruchtel et

al. (1996) is withdrawn. Applicant's arguments have been considered but are moot in view of the new ground(s) of rejection.

8. The rejection of claim 27 under 35 U.S.C. 103(a) as being unpatentable over Berglund et al. as applied to claim 1 and further in view of Bambara et al. (1975, previously cited), Bloch (1999), Crane et al. (1992 previously cited), and Asteriadis et al. (1976 previously cited) is withdrawn. Applicant's arguments have been considered but are moot in view of the new ground(s) of rejection.

Rejections that are Maintained

Claim Rejections Maintained - 35 USC § 102

9. The rejection of claims 1, 2, 6-8, 10-14, 16-21, 25, and 28 under 35 U.S.C. 102(b) as being anticipated by Berglund et al. (United States Patent 6,090,288 issued July 18, 2000) is maintained. Applicant's arguments have been fully considered but they are not persuasive.

First it is acknowledged that Applicant is correct in conveying that the section of Berglund et al. cited by Examiner at column 2 lines 33-44 is not within the scope of the disclosure of Berglund et al., as such is stated by Berglund et al. themselves. Thus this citation is withdrawn as support for the rejection. However, this withdrawal does not overcome the rejection, as follows.

Applicant argues that claim 14 of Berglund et al. cannot meet the standard required for anticipation of Applicant's claims, as the alternate recitation of “increasing

pH' in claim 14 by Berglund et al. cannot be interpreted as increasing pH in the absence of a substantial increase in salt. Examiner respectfully disagrees. The text of claim 14 is provided:

> "14. Process according to claim 2, characterized in that nucleic acid is separated off by adsorption to the ion exchanger and that adsorbed nucleic acid subsequently is eluted by
> a. an agent which breaks hydrogen bonds, and/or,
> b. salt and/or
> c. increasing of pH so that the ligands are completely or partially unloaded."
> A direct read of the claim reveals that the claim encompasses the embodiment

Here Burglund et el. claim that absorbed nucleic acid is eluted by increasing pH without salt. This is due to the logical construction of the three choices of a. and/or b. and/or c. in the claim. Berglund et al. clearly claim the use of any one of the three choices by itself and in all possible combinations with the other two choices. Thus elution can be accomplished with an increase in pH by itself and without salt. As there is no salt, inherently there can be no increase in salt and there is inherently no need for desalting, thus meeting these limitations of instant claim 1, particularly step c).

Applicant then argues that the disclosure of Berglund et al. provide examples with increase in salt concomitant with increase in pH. Regardless, claim 14 of Berglund et al. reveals that Berglund et al. understood and specifically expressed that the pH could be a sole changing factor in elution of nucleic acids. Furthermore, Berglund et al. provide teachings in support of claim 14 as follows:

> "The solution (including the eluent) applied on the anion exchanger is typically aqueous consisting of water, possibly comprising a liquid miscible with water, for example ethanol, methanol, acetonitrile, mixtures miscible with water etc. The solution may also be any of the above mentioned organic solvents. The solutions, especially eluents, often contain salts, buffer substances, substances

which break hydrogen bonds etc. " (see column 3 lines 47-54), and go on to teach:

"Use of denaturating/hydrogen bond-breaking agents is above all applicable for separation of nucleic acid and can easily be combined with a salt gradient (=conductivity gradient) **or** changes of pH.

Generally speaking, if the adsorption involves a significant portion of hydrogen bonds, desorption can be performed at relatively low ionic strengths if agents which break hydrogen bonds (e.g. free ligand) are included in the desorption solution or by raising pH so that the ligand is unloaded and the hydrogen bonds are impaired" (emphasis by Examiner, see column 8 lines 32-41 and noting that changes in pH alone can be used and low ionic strength inherently means low salt and low amounts of other sources of ions).

Berglund et al. disclose that although eluents "often" contain salts that eluents do not always contain salts. Berglund et al. further disclose that unloading the ligand can be accomplished by raising pH but keeping the ionic strength low which inherently means low amounts of salt ions and other ions. Thus Berglund et al., as they recite in claim 14 and as they teach in the specification, disclose that elution of nucleic acids can be achieved with an increase in pH without salt or without a substantial increase in salt, with the result that subsequent desalting is not required. Thus the rejection is maintained.

New Rejections

New Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claims 1-4 and 6-28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Johansen et al. (United States Patent 6,441,152 issued August 27, 2002 and filed December 8, 1999), Gilar et al. (2000), Fruchtel et al. (1996, previously cited), and Ferreira et al. (2000, previously cited).

Regarding claims 1-3, 10, and 12 Johansen et al. teach methods of separating a target oligonucleotide from an impurity, in a mixture comprising said target oligonucleotide and said impurity, using a titratable anion exchange composition (entire patent), comprising the steps:

a) binding said target oligonucleotide which is a synthetic DNA probe to said titratable anion exchange composition which can be polyethylene imine (PEI) in the presence of a solution having a first pH which can be pH 7.2 and up to 0.8M NaCl (see Example 9 section *ii. pH Effects*); and

b) passing solution through said titratable anion exchange composition with target oligonucleotide bound thereon, wherein the pH of said elution solution is increased over time to a pH higher which can be pH 8.3 (see Example 9 section *ii. pH Effects*) than said first pH thereby to elute said target oligonucleotide, wherein said impurity elutes at a different pH than said target oligonucleotide, and wherein

c) the elution solution does not substantially increase its salt concentration over time such that subsequent desalting of the eluded eluted oligonucleotide is not required by teaching that less salt at the higher pH of pH 8.3 and up to 0.1 NaCl which is a decrease in salt that is required to release the bound DNA and further teaching:

> "These results can be correlated with the number of expected positively charged functional groups of the PEI support. The higher capacity at pH 7.3 indicates that

> the support is more highly charged (higher charge density) under these conditions. However, at pH 8.3, the pH of the solution is approaching the pK of the secondary amine of the PEI and therefore the support begins to become neutralized (fewer positive charges per unit area). With fewer positive charges, each bead has a lower affinity for the negatively charged oligodeoxynucleotides. Therefore, less salt is required to disrupt the electrostatic interactions and thereby release the DNA into the solution" (see column 33 line 39 to column 34 line 3 in Example 9 section *ii. pH Effects*).

Further regarding claim 1, Johansen et al. at least teach that PNA oligonucleotides will be separated from DNA oligonucleotides as the PNA oligonucleotides will not bind under the first pH and salt conditions and thus PNA and DNA oligonucleotides will elute from a mixture of these (see Example 9 sections i and ii).

Johansen et al. teach separation of oligonucleotides but do not specifically teach separation of 5' O-protected target oligonucleotides from failed sequences.

Regarding claims 4 and 6-8, Johansen et al. teach supports which are matrices with synthetic polymers which include styrene DVB (styrene-divinyl benzyne) conjugated through cross-linking of covalent attachment to an insoluble body support which polymers are anion exchange materials of charged functional groups (see column 7 lines 8-31).

Regarding claim 9, Johansen et al. teach PEI-Silica (gel) beads (see column 29 lines 45-47).

Regarding claims 14, 15, and 27, Johansen et al. teach that ammonium hydroxide can be used to release DNA (see column 37 line 36) and teach that nucleic acids bound at pH 7.6 which is about pH 8 can be released at pH 10.7 (see column 39

line65 to column 40 line 4). Furthermore, Johansen et al. teach that their methods, unlike prior methods, can be optimized through routine optimization to easily fix binding and releasing conditions o and thus teach that variations in pH and choice of buffers were obvious:

> "Variable factors which will most affect electrostatic binding will involve modulation of one or both the pH and/or ionic strength. The pH is an important factor since it may affect the charge density on the matrix as well as the net charge of the nucleic acid" (see column 17 lines 28-30) and
>
> "Since pH and ionic strength are factors to be considered in both stringency and electrostatic binding and since the electrostatic binding conditions are broad as compared with optimized stringency, it should always be possible to easily fix the electrostatic binding conditions and then optimize probe discrimination by modulation of other stringency factors. In this respect, the methods of this invention are far superior to current methods known in the art (e.g. Arnold et al, U.S. Pat. No. 5,599,667). Aided by no more than routine experimentation, those of skill in the art will easily be able to harmonize the electrostatic binding conditions and suitable hybridization conditions for performing an assay" (see column 18 lines 2-34).

Regarding claim 16, Johansen et al. teach oligonucleotides between about 8 to about 40 nucleotides in length (see Table 1).

Regarding claim 17, Johansen et al. teach oligonucleotides which are PNA's elute at the lower pH than oligonucleotides which are DNA's and teach PNA's and DNA's of varying length (see Example 9 sections i and ii, and see Table 1).

Regarding claim 24, Johansen et al. teach concentrating the beads after binding the mixture and removing the supernatant which necessarily concentrates the bound mixture washing steps prior to elution (see column 39 lines 65 and 66).

Regarding claim 19, Ferreira et al. teach removal of metal salt impurities by chelation with EDTA (see first two sentences on p. 385).

Further regarding claim 24, Ferreira et al. teach "AEC [Anion Exchange Chromatography] has been used as an initial purification step to capture and to concentrate plasmid DNA" (see p. 386, 2nd paragraph, 2nd sentence under the section *Strategies for purifying supercoiled plasmid DNA*).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the methods of anion exchange of Johansen et al. by using an anion exchange composition to concentrate a target oligonucleotide and using EDTA to remove metal salt as suggested by Ferreira et al. with a reasonable expectation of success. The motivation to do so is provided by Ferreira et al. who teach the concentration of a target oligonucleotide, a target DNA plasmid, by AEC and the teaching and the teaching of Johansen et al. for the separation of oligonucleotides by anion exchange and that removal of metal salts prevents carryover during chromatography (see last sentence on p. 384 continued to p. 385). Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

Regarding claim 25, Johansen et al. teach washing steps prior to elution (see column 39 line 66 to column 40 line 1).

Regarding claim 28, Johansen et al. teach as noted above and further teach binding mixtures of PNA and DNA oligonucleotides and different pH conditions for elution of these (entire patent, especially Example 13).

Regarding claims 1, 18, 20-23, and 26, Gilar et al. teach methods of separating a target oligonucleotide from an impurity which can be failed sequences, in a mixture comprising said target oligonucleotide and said impurity of failed sequences (entire article, especially the Abstract), using a titratable reverse phase composition, comprising the steps:

a) binding said target oligonucleotide which is 5'-O protected including 5'O-demethoxytrityl protected (see paragraphs 3 and 4 on p. 168) to said titratable reverse phase composition in the presence of a solution having a first pH of ph 8.0 (see Table 1); and

b) passing solution through said titratable reverse phase composition with target oligonucleotide bound thereon, wherein the pH of said elution solution is increased over time to a pH higher of pH 11.3 than said first pH thereby to elute said target otigonucleotide (see Table 1), wherein said impurity of failed sequences elutes at a different pH than said target oligonucleotides of "trityl off" (see 3rd paragraph on p. 168), and

b 1) further comprising a step prior to elution of cleaving the 5'-O trityl group "on column" (see last two sentences of the 3rd paragraph on p. 168),

c) the elution solution does not substantially increase its salt concentration over time such that subsequent desalting of the eluded eluted oligonucleotide is not required (see table 1).

Further regarding claim 1, Gilar et al. teach methods of reverse phase which operate by anion exchange in the mobile phase between the sample compounds and components of the mobile pahse, and teach anion exchange chromatography with ion pairing, but do not specifically teach anion exchange support chromatography for separation of 5' O-protected target olignucleotides from failed sequences and other impurities.

Further regarding claim 23, Gilar et al. teach the acidic solution for cleaving on column which is trifluoracetic acid (see last two sentences of the 3rd paragraph on p. 168) which is a type of acetic acid and teach acidic acid (see 3rd paragraph on p. 170) for cleavage of the 5'-O protecting group.

Even further regarding claim 23, Fruchtel et al. teach that acid condition cleave 5'-O-trityl protecting group including acetic acid (see 2nd sentence on page 20: " . . . the trityl anchoring bond can be cleaved by very weak acids such as acetic acid"). Thus in view of these teachings it would have been obvious to use acetic acid to cleave 5'O-trityl protecting groups.

Regarding claim 11, Gilar et al. teach separation of phoshorotioate oligonucleotides (see 4th paragraph on p. 170).

Regarding claim 13, Gilar et al. teach a linear gradient with gradient slope of 0.25% of acetonitrile per mL of mobile phase (see 5th sentence on p. 175).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the chromatographic methods of Johansen et al. by binding, separating, and cleaving 5' O- protected target oligonucleotides as suggested by Gilar et al. for chromatographic methods with a reasonable expectation of success. The motivation to do so is provided by Johansen et al. and Gilar et al. who both teach types of anion separation of oligonucleotides by increasing pH over time. Gilar et al. also teach that their method: ". . . eliminates the need for further desalting" (see last sentence of the Abstract). And likewise, Johansen et al. teach that use of pH elution reduces salt in the eluted oligonucleotides. Furthermore, Johansen et al. teach that their methods can routinely and easily be optimized. This provides further motivation to implement the binding, separating, and cleaving of 5' O- protected target oligonucleotides as suggested by Gilar et al. into the methods of Johansen et al. Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

12. Claims 3, 4, and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Berglund et al. as applied to claim 1 and 8 above and previously, and further in view of Bloch (United States Patent 5,856,192 issued January 5, 1999, previously cited) and Asteriadis et al. (1976, previously cited).

Applicant correctly notes that Examiner cited teaching of Bloch for elements of claims 1, 2, and 28 which were already rejected as being anticipated by the teachings of Berglund et al. Examiner did this to point out that Bloch teaches many of the same

elements as Berglund et al. and thus this would combining the teachings of Bloch and Berglund et al. obvious to one of ordinary skill in the art. To provide further clarification, the rejection is presented below with edits regarding claims 1, 2, and 28.

Berglund et al. teach as noted above.

Berglund et al. teach where the compositions comprise amines but do not specifically teach where the compositions comprise polyethyleneimine, polyimizadole, polyhistidine or polylysine.

Regarding claims 3 and 9 and similar to the teachings of Berglund et al. also regarding claims 1, 2, and 28, Bloch teaches methods of separating a target oligonucleotide from an impurity, in a mixture comprising said target oligonucleotide and said impurity, using a titratable anion exchange composition, (entire patent especially the Title and Abstract) comprising the steps:

a) binding said target oligonucleotide to said titratable anion exchange composition which can comprise polyethyleneimine (see claim 7) or which can comprise polylysine (see column 6 lines 63-66) in the presence of a solution having a first pH; and

b) passing a solution through said titratable anion exchange composition with target oligonucleotide bound thereon, wherein the pH of said elution solution is changed over time through a pH gradient (column 17 line 16) thereby to elute said target oligonucleotide, wherein said impurity elutes at a different pH than said target oligonucleotide and wherein

c) the elution solution is substantially free from metal salts such that subsequent desalting of the eluted oligonucleotide is not required by teaching

> "Solvents for salt-gradient anion-exchange separation of nucleic acids, especially double-stranded DNA and especially by liquid chromatography, are improved by replacing NaCl [a metal salt] as the eluting salt with any of a wide range of alkyl ammonium salts [non-metal salts] and can be used to elute nucleic acids in strict order of increasing length, with reduced sensitivity to elution temperature and salt concentration" (see 1st sentence of the Abstract);

Bloch et al. also teach that there are several formats for anion-exchange chromatography which were commonly known to practioners of separation art; and that a pH gradient format is a separate elution format from a salt gradient format and thus at least suggest elution with a pH gradient and without a salt gradient, that is, without any substantial increase in salt concentration (see column 17 lines 9-22).

Regarding claims 1 and 28, Bloch teaches pH gradients but does not specifically teach increasing the pH over time.

Regarding claim 4, Bloch teaches that elution is done without metal salts and thus necessarily is substantially free of metal salts (see 1st sentence of the Abstract).

Berglund et al. teach separating oligonucleotides from impurities using a titratable anion composition comprising amines by increasing pH over time. Berglund et al. do not specifically teach where the amines are polyethyleneimine or polylysine. Bloch teaches separating oligonucleotides from impurities using a titratable anion composition comprising polyethyleneimine or polylysine by changing pH over time. Bloch does not specifically teach increasing the pH over time. Because both Berglund et al. and Bloch teach separation using amines, it would have been obvious to one skilled

in the art to substitute the amines which are polyethyleneimine or polylysine as taught by Bloch for the amines taught by Berglund et al. in order to achieve the predictable result of a method of separating oligonucleotides from impurities using a titratable anion composition comprising amines which are polyethyleneimine or polylysine by increasing pH over time. Also, motivation to do so is provided by Bloch who teach that their: "Anion-exchange chromatography with these solvents [without metal salts] is well suited for identification of DNA fragments on the basis of size, with greater accuracy, precision, and resolvable size range than often is possible with gel electrophoresis" (see 2nd sentence of the Abstract). Furthermore, Bloch teaches that use of non metal salts, which are di-, tri-, or tetra-alkylammonium cations in combination with a variety of anions, in solvents for chromatography (see column 8 lines 34-52) improves anion-exchange separation and analysis of nucleic acids (see column 9 lines 48-50). Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

13. Claims 15, 22, 23, 26, and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Berglund et al. as applied to claim 1 above and previously and further in view of Bloch, Asteriadis et al. (1976, previously cited), Gilar et al. (2000), and Fruchtel et al. (1996, previously cited).

Claims 15 and 27

Berglund et al. and Bloch teach as noted above.

Regarding claim 27 in part, Bloch suggest that increasing pH from between about 4 and about 9 (see column 12 lines 14-19), which includes pH 8, to about pH 11 removes the charge from DEAE and PEI which then removes the anionically bound components as further taught:

> "When a liquid contacting the anion-exchange solid is an aqueous solvent of pH below about 9 to 11 the nitrogen atoms of DEAE and PEI are protonated and therefore positively charged" (see column 11 lines 48-53).

Regarding claim 27, Berglund et al. and Bloch in combination teach the method of claim 1 as given above, teach polyethyleneimine as give above and alos teach anion exchange with polystyrene-divinylbenzene resins, teach elution free of metal salts and where the salt concentration does not substantially increase as give above; but do not specifically teach a solution of NH_4HCO_3 or NH_4OH.

Regarding claims 15, Berglund et al. and Bloch do not specifically teach a solution of NH_4HCO_3 or NH_4OH.

Regarding claims 15 and 27, Asteriadis et al. teach a method using a solution of NH_4OH with polystyrene anion exchangers (see Abstract and p. 67, 1st sentence).

Further regarding claim 27 in part, Gilar also teach an initial pH 8.0 of and an eluting pH of 11.3 (see Table 1).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the methods of Berglund et al. and

Bloch using low salt eluents and a polystryrene anion exchanger with an increase in pH from about pH 8 to about pH 11 by using the low salt eluent of NH_4OH as suggested by Asteriadis et al. with a reasonable expectation of success. The motivation to do so is provided by Asteriadis et al. who teach the usefulness of a method of low salt elution with a polystyrene anion exchanger and an eluent of NH_4OH for purification of oligonucleotides and the teaching of Berglund et al. Bloch for the separation of oligonucleotides with other low salt eluents with polystyrene anion exchanger. Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

Claims 22, 23, and 26

Berglund et al. and Bloch et al. teach as noted above.

Regarding claims 22, 23, and 26, Bloch does not specifically teach 5'-O-protected oligonucleotides.

Regarding claims 22, 23, and 26, Berglund et al. further teach a method wherein a target oligonucleotide is 5'-O-protected, is 5'-O-trityl protected, but do not specifically teach where there is a sufficient amount of an acidic solution to cleave said 5'-O-trityl protecting group from a target oligonucleotide prior to elution, and where acidic solution comprises aqueous acetic acid.

Regarding claims 22, 23, and 26 and also teaching similar to Berglund et al. in regards to claims 1, 18, 20, and 21, Gilar et al. teach methods of separating a target oligonucleotide from an impurity which can be failed sequences, in a mixture comprising

said target oligonucleotide and said impurity of failed sequences (entire article, especially the Abstract), using a titratable reverse phase composition, comprising the steps:

a) binding said target oligonucleotide which is 5'-O protected including 5'O-demethoxytrityl protected (see paragraphs 3 and 4 on p. 168) to said titratable reverse phase composition in the presence of a solution having a first pH of pH 8.0 (which I about pH 8see Table 1); and

b) passing solution through said titratable reverse phase composition with target oligonucleotide bound thereon, wherein the pH of said elution solution is increased over time to a pH higher of pH 11.3, which is about pH 11, than said first pH thereby to elute said target oligonucleotide (see Table 1), wherein said impurity of failed sequences elutes at a different pH than said target oligonucleotides of "trityl off" (see 3rd paragraph on p. 168), and

b 1) further comprising a step prior to elution of cleaving the 5'-O trityl group "on column" (see last two sentences of the 3rd paragraph on p. 168),

c) the elution solution does not substantially increase its salt concentration over time such that subsequent desalting of the eluded eluted oligonucleotide is not required (see table 1).

Further regarding claim 1, Gilar et al. teach methods of reverse phase which operate by anion exchange in the mobile phase between the sample compounds and components of the mobile phase, and teach anion exchange chromatography with ion pairing, but do not specifically teach anion exchange support chromatography for

separation of 5' O-protected target olignucleotides from failed sequences and other impurities.

Regarding claim 23, Gilar et al. teach the acidic solution for cleaving on column which is trifluoracetic acid (see last two sentences of the 3rd paragraph on p. 168) which is a type of acetic acid and teach acidic acid (see 3rd paragraph on p. 170) for cleavage of the 5'-O protecting group.

Further regarding claim 23, Fruchtel et al. teach that acid condition cleave 5'-O-trityl protecting group including acetic acid (see 2nd sentence on page 20: " . . . the trityl anchoring bond can be cleaved by very weak acids such as acetic acid"). Thus in view of these teachings it would have been obvious to use acetic acid to cleave 5'O-trityl protecting groups.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the chromatographic methods of Berglund et al. for binding, separating, and cleaving 5' O- protected target oligonucleotides as suggested by Gilar et al. with a reasonable expectation of success. The motivation to do so is provided by Berglund et al. and Gilar et al. who both teach types of anion separation of oligonucleotides by increasing pH over time. Gilar et al. also teach that their method: ". . . eliminates the need for further desalting" (see last sentence of the Abstract). And likewise, Berglund et al. teach that use of pH elution reduces salt in the eluted oligonucleotides. Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

14. Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable Berglund et al. as applied to claim 1 above and previously and further in view of Ferreira et al. (2000, previously cited).

Berglund et al. teach as noted above.

Berglund et al. do not specifically teach a method wherein anion exchange concentrate the target oligonucleotide.

In regards to claim 24, Ferreira et al. teach "AEC [Anion Exchange Chromatography] has been used as an initial purification step to capture and to concentrate plasmid DNA" (see p. 386, 2nd paragraph, 2nd sentence under the section *Strategies for purifying supercoiled plasmid DNA*).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the anion exchange methods of Berglund et al. by using an anion exchange composition to concentrate a target oligonucleotide as suggested by Ferreira et al. with a reasonable expectation of success. The motivation to do so is provided by Ferreira et al. who teach the concentration of a target oligonucleotide, a target DNA plasmid, by AEC and the teaching and the teaching of Berglund et al. for the separation of oligonucleotides by anion exchange. Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

Conclusion

15. No claim is free of the prior art.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark Staples whose telephone number is (571) 272-9053. The examiner can normally be reached on Monday through Thursday, 9:00 a.m. to 6:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Mark Staples/
Examiner
Art Unit 1637
September 5, 2009